

## ***Cryptosporidium* Sample Results Acceptability Checklist for the LT2 Rule**

No.	Factor	Check sample results to verify that:	U
<b>General Requirements</b>			
1	Sample condition upon receipt	The sample temperature upon receipt for the sample was between 0°C and 8°C, and the sample was not frozen	
2	Sample volume	The volume analyzed for the sample was at least 10 L OR at least 2 mL of packed pellet volume was analyzed OR 2 filters were used and clogged and 100% of the volume filtered was analyzed	
<b>Holding Time Requirements</b>			
3	Sample collection	The elution step for the sample was initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field)	
4	Sample processing	The sample was processed (eluted, concentrated, purified, and applied to the slide) in one working day	
5	Sample drying	The slide(s) for the sample were stained within 72 hours of application of the sample to the slide	
6	Sample examination	The slide(s) for the sample were examined within 7 days of staining	
<b>Quality Control (QC) Sample Requirements</b>			
7	QC batch	The ongoing precision and recovery (OPR) and method blank samples associated with the sample are associated with no more than 20 field and matrix spike samples and no more than a week of samples	
8	Method blank results	The method blank sample for the QC batch was acceptable (no <i>Cryptosporidium</i> oocysts or potentially interfering materials were found in the method blank)	
9	OPR sample results	The OPR sample for the QC batch was acceptable (recovery was at least 24%)	
10	Spike levels for OPR	The associated OPR was spiked with no more than 500 oocysts	
11	Spike materials for OPR	The spiking suspension used for the associated OPR was flow-cytometer sorted	
<b>Staining Control Requirements</b>			
12	Staining control frequency	A positive and negative staining control slide is associated with the slide for this sample	
13	Positive staining control result	The positive staining control for this sample is acceptable (it contained oocysts within the expected range and at the appropriate fluorescence for both FITC and DAPI)	
14	Negative staining control result	The negative staining control for this sample is acceptable (it did not contain any oocysts)	
<b>Matrix Spike (MS) Sample Requirements</b>			
15	Sample volume	The same sample volume was analyzed for the MS sample and the associated, unspiked field sample	
16	Method version	The same method version (filter, IMS, staining kit) was used for the MS sample and the associated, unspiked field sample	
17	Spike amount for MS	The MS sample was spiked with no more than 500 oocysts	
18	Spike materials for MS	The spiking suspension used for the MS was flow-cytometer sorted	